

A reassessment of *Hourangia cheoi* from Yunnan, China

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ABSTRACT—A recent collection of *Hourangia cheoi* (Boletaceae, Boletales) from near the type locality in Yunnan Province, southwestern China, is described in detail and illustrated, and its nrITS and nrLSU rDNA sequences are included in molecular phylogenetic analyses. Comparisons with closely related xerocomoid taxa are also provided.

KEY WORDS—biogeography, ectomycorrhizal fungi, taxonomy, *Xerocomus*

Introduction

During repeated mycological field expeditions carried out by the first author during 2011–13 in central, southwestern, and southeastern China (Beijing, Shaanxi, Yunnan, and Guangdong Provinces), several new or noteworthy fungal species were recorded and documented (Gelardi et al. 2012, 2013, 2014, 2015a,b; Vizzini et al. 2012, 2014; Battistin et al. 2014, Gelardi 2014, Picillo & Gelardi 2014). Amongst the several bolete species found in the surveyed areas, a single specimen with a xerocomoid habit was collected near Dali, Yunnan, and later identified from morphological and molecular analyses as *Hourangia cheoi*.

Chiu (1948) originally described the species in the genus *Boletus* Fr. In the same paper the Chinese author described *Boletus punctilifer*; both species were subsequently illustrated (Chiu 1957) and later combined in *Xerocomus* Quél. (Tai 1979). However, Zhu et al. (2015) reduced *B. punctilifer* to synonymy under *B. cheoi* after examining numerous specimens, including the holotypes of both names.

As a result of intensive research on Chinese xerocomoid boletes, Zhu et al. (2015) erected a new genus *Hourangia* based on *Boletus cheoi*, including three other species originally described as *B. microcarpus* Corner, *B. nigropunctatus* W.F. Chiu, and *Phylloporus pumilus* M.A. Neves & Halling. *Hourangia* is related to *Xerocomus* s.str. and sister to *Phylloporus* Quél. (Wu et al. 2014, Zhu et al. 2015, Henkel et al. 2016), and despite the phylogenetic and morphological affinities with these genera, it is readily distinguished by a finely squamulose pileus surface, tubes that are three to seven times longer than the thickness of pileus context, whitish basal mycelium, context turning blue then reddish and finally fading to blackish–brown on exposure, a bacillate spore ornamentation (visible only with a scanning electron microscope), a “*Phylloporus*–type” hymenophoral trama, ectomycorrhizal association with *Fagaceae*, *Pinaceae*, and *Dipterocarpaceae*, and its eastern and southeastern Asian distribution (Zhu et al. 2015; pers. obs.).

The aim of the present contribution is to provide new insights and iconography on *Hourangia cheoi*.

Materials & methods

Collection site and sampling

The specimen was collected in the surroundings of Dali, Yunnan Province, China, and is deposited in ZT (Thiers 2017) and in the personal herbarium of Matteo Gelardi (MG). Author citations follow Index Fungorum (www.indexfungorum.org/authorsoffungalnames.htm).

Morphological studies

Macroscopic descriptions, habitat notations, and associated plant communities were based upon detailed field notes from fresh basidiomes. Color terms in capital letters (e.g. Myrtle Green, pl. VIII) are from Ridgway (1912). The specimen was photographed in habitat using a Nikon D3100 camera. Micro–morphologic features were observed from dried material; sections were rehydrated in water, 5% potassium hydroxide (KOH), or ammoniacal Congo Red. Structures and anatomical features were observed and measured in Congo Red; color and pigments were observed in KOH. Measurements were determined at 1000× with a calibrated ocular micrometer (Nikon Eclipse E200 optical light microscope). Basidiospores were measured from the mature hymenophore; dimensions are given as (minimum–) average $\pm$ standard deviation (–maximum), Q = length/width (L/W) ratio with the extreme values in parentheses, Q_m = average quotient (L/W ratio) $\pm$ standard deviation, and average spore volume was approximated as a rotation ellipsoid ($V = (\pi \cdot L \cdot W^2) / 6 \pm$ standard deviation). The notation [n/m/p] indicates that measurements were made on “n” randomly selected basidiospores from “m” basidiomes of “p” collections. The width of each basidium was measured at the widest part, and the length was measured from

the apex (sterigmata excluded) to the basal septum. Metachromatic, cyanophilic, and iodine reactions were tested by staining the basidiospores in Brilliant Cresyl blue, Cotton blue and Melzer's reagent, respectively. Line drawings of microstructures were made freehand based on photomicrographs of rehydrated material.

DNA extraction, PCR amplification and sequencing

Total DNA was extracted from the dry specimen (MG484) with the DNeasy Plant Mini Kit (QIAGEN, Milan, Italy) according to the manufacturer's instructions. PCR primers ITS1F and ITS4 (White et al. 1990, Gardes & Bruns 1993) for the nrITS region, LR0R, LR1, LR5 and LR7 (Vilgalys & Hester 1990, Cubeta et al. 1991, Van Tuinen et al. 1998) for the nrLSU region, were employed for PCR amplification and sequencing purposes. The PCR protocol comprised a hot start at 95°C (5 min), followed by 35 cycles of 94°C (45 s), 54°C (30 s), and 72°C (45 s), and a final 72°C step (10 min). PCR products were checked in 1% agarose gels before purification and sequencing. Chromatograms were searched for putative sequencing reading errors. The sequences are deposited in GenBank and their accession numbers are included in Figs 1, 2.

Sequence alignment, dataset assembly and phylogenetic analyses

The sequences obtained in this study were checked and assembled using Geneious v. 5.3 (Drummond et al. 2010) and compared to those available in GenBank using the Blastn algorithm. Based on the BLASTn results (with sequences selected based on greatest similarity) and the phylogenetic analysis by Zhu et al. (2015), sequences were retrieved from GenBank and UNITE (<http://unite.ut.ee/>) databases for the comparative phylogenetic analysis. Alignments were generated for each single LSU and ITS dataset using MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The two alignments were then imported into MEGA v. 6.0 (Tamura et al. 2013) for manual adjustment. The best-fit models were estimated by the Bayesian Information Criterion (BIC) using jModelTest v. 2.1.7 (Darriba et al. 2012) to provide a substitution model for each single alignment. GTR+G models were chosen for both the ITS and LSU alignments.

Phylogenetic analyses were performed using the Bayesian Inference (BI) and Maximum likelihood (ML) approaches. *Phylloporus rhodoxanthus* (JQ003654), *Xerocomus ferrugineus* (JQ003657) and *X. subtomentosus* (KC215201) were chosen as the outgroup taxa in the ITS analysis (Fig. 1); and *Phylloporus imbricatus* (KF112398), *P. luxiensis* (KF112490), *P. pelletieri* (AF456818), *P. rubrosquamosus* (KF112391), "*Xerocomus perplexus*" (JQ003702), and *X. subtomentosus* (KC215222) in the LSU analysis (Fig. 2), following Zhu et al. (2015). Bayesian and ML inferences were performed online using the CIPRES Science Gateway website (Miller et al. 2010) and both methods were implemented as single software usage. BI phylogeny using Monte Carlo Markov Chains (MCMC) was carried out with MrBayes v. 3.2.2 (Ronquist et al. 2012). Four incrementally heated simultaneous MCMC were run over 10 M generations. Trees were sampled every 1000 generations resulting in an overall sampling of 10,001 trees. The first 2500 trees (25%) were discarded as burn-

in. For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). Branch lengths were estimated as mean values over the sampled trees. ML estimation was performed through RAXML v. 7.0.4 (Stamatakis 2006) with 1000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMA algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the 'f a' option of RAXML and '-x 12345' as a random seed to invoke the novel rapid bootstrapping algorithm. Only BPP values >0.75 and/or MLB values >50% are reported in the resulting trees.

Results

Both Bayesian and Maximum Likelihood analyses produced the same topology; only the BI trees with both BPP and MLB values are shown (Figs 1, 2).

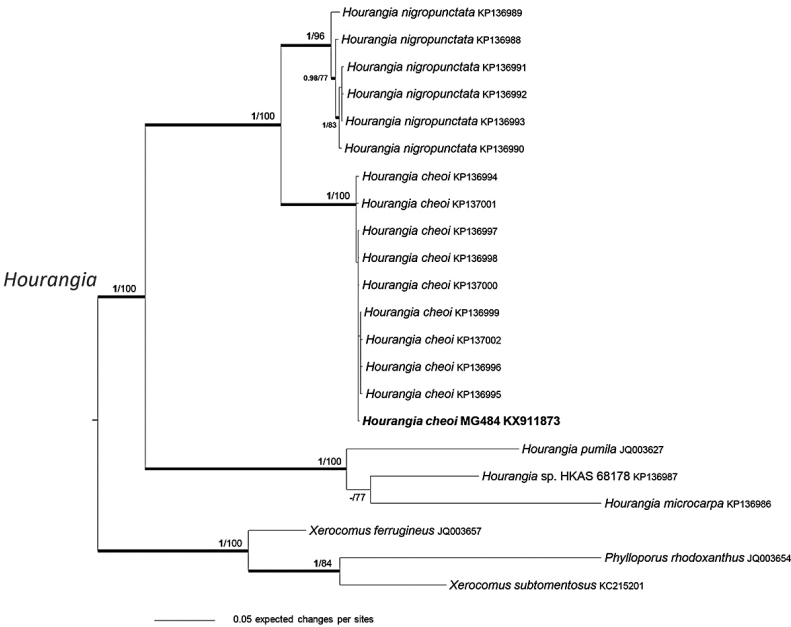


FIG. 1. Bayesian phylogenetic analysis based on the ITS sequences of *Hourangia* species and related taxa. Bayesian posterior probabilities (BPP; in bold) ≥ 0.75 and Maximum Likelihood bootstraps (MLB) $\geq 50\%$ are shown on the branches. Thickened branches indicate BPP ≥ 0.95 and MLB $\geq 70\%$. The GenBank/UNITE number is given for each sequenced taxon. The new *H. cheoi* sequence is in bold.

The ITS data matrix comprised a total of 22 sequences (including 21 from GenBank); the alignment comprised 1160 characters and contained 526 (45.3%) variable sites, of which 284 (24.5%) were parsimony informative. The LSU matrix consisted of 34 sequences (including 33 from GenBank); the alignment comprised 830 characters and contained 144 (17.3%) variable sites, of which 110 (13.2%) were parsimony informative.

Both in the ITS and LSU analyses, our collection and the *Hourangia cheoi* sequences retrieved from GenBank form a strongly supported clade (BPP = 1 and MLB = 100 in the ITS analysis; BPP = 0.95 and MLB = 95 in the LSU analysis).

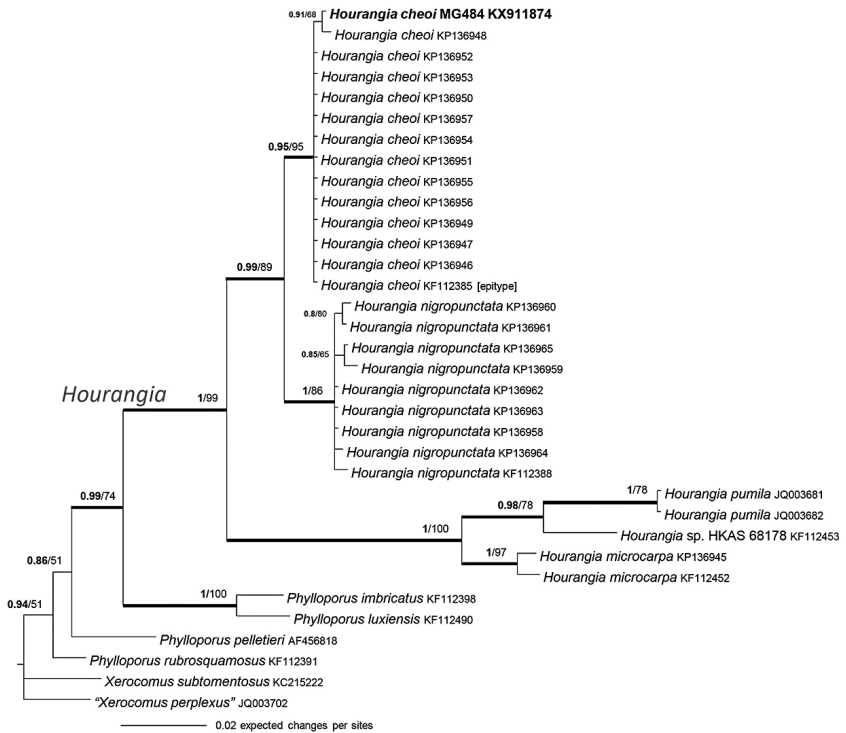


FIG. 2. Bayesian phylogenetic analysis based on the LSU sequences of *Hourangia* species and related taxa. Bayesian posterior probabilities (BPP; in bold) ≥ 0.75 and Maximum Likelihood bootstraps (MLB) $\geq 50\%$ are shown on the branches. Thickened branches indicate BPP ≥ 0.95 and MLB $\geq 70\%$. The GenBank number is given for each sequenced taxon. The new *H. cheoi* sequence is in bold.

Taxonomy

Hourangia cheoi (W.F. Chiu) Xue T. Zhu & Zhu L. Yang,

Mycol. Progr. 14: 37 [4 of 10] (2015).

FIGS 3, 4

MYCOBANK MB 810696

≡ *Boletus cheoi* W.F. Chiu, Mycologia 40(2): 215 (1948).

≡ *Xerocomus cheoi* (W.F. Chiu) F.L. Tai, Syll. Fung. Sinicorum: 813 (1979).

= *Boletus punctilifer* W.F. Chiu, Mycologia 40(2): 216 (1948).

≡ *Xerocomus punctilifer* (W.F. Chiu) F.L. Tai, Syll. Fung. Sinicorum: 814 (1979).

BASIDIOMES medium-small. **PILEUS** 4.3 cm diam., convex-pulvinate, regularly shaped, moderately fleshy, soft; margin straight to faintly wavy, curved downwards, not or only slightly extending beyond the tubes; surface matte, dry, very finely squamulose, not cracked; cuticle evenly dark ochraceous (Buckthorn Brown, pl. XV; Sayal Brown, pl. XXIX); slowly darkening to dull brownish-black (Warm Sepia, pl. XXIX) on handling or when injured, especially at the edge of squamules; subcuticular layer whitish (White, pl. LIII). **TUBES** somewhat broad and decidedly longer than the thickness of pileus context (≤ 1.0 cm long), adnate, olive yellow (Javel Green, pl. V), turning blue (Myrtle Green, pl. VIII) when cut. **PORES** forming a slightly convex or ascendant surface, simple, not radially arranged, prominently wide and angular (≤ 1 mm in diam.), concolorous with the tubes and turning blue (Myrtle Green, pl. VIII) on bruising or when injured and finally fading to brownish (Snuff Brown, pl. XXIX), without innate rusty brown stains at the orifice. **STIPE** 5.3×0.6 cm, slightly longer than pileus diameter, central, solid, firm, dry, faintly curved, cylindrical but slightly swollen at the base, ending with a short taproot at the very base; surface finely longitudinally fibrillose, devoid of reticulum or any other kind of ornamentation, veils absent; beige-ochraceous (Cream Buff, pl. XXX) throughout but paler (Cartridge Buff, pl. XXX) towards the base, unchangeable when pressed; basal mycelium whitish (White, pl. LIII), rhizomorphs brownish (Dresden Brown, pl. XV). **CONTEXT** soft textured and thin in the pileus (≤ 0.4 cm thick in the central zone), a little more fibrous in the stipe, whitish (White, pl. LIII) throughout but pale yellow (Martius Yellow, pl. IV) above the hymenophore; turning light blue (Pale Methyl Blue; Light Cerulean Blue, pl. VIII; Sky Blue, pl. XX) in the pileus and stipe/pileus connecting zone when exposed to air but changing within twenty seconds to bright flesh pink (Buff Pink, Congo Pink, pl. XXVIII) and eventually fading to grayish (Light Mouse Gray, pl. LI) in patches, elsewhere in the stipe directly turning to pinkish-red (Congo Pink, Japan Rose, Terra Cotta, pl. XXVIII) after cutting, especially towards the base, then fading to dirty grayish (Mouse



FIG. 3. *Hourangia cheoi* (MG484).
Basidiome in habitat. Scale bar = 2 cm. Photo by M. Gelardi.

Gray, pl. LI) or grayish-black (Blackish Mouse Gray, Sooty Black, pl. LI); sub-hymenophoral layer yellowish (Martius Yellow, pl. IV); exsiccate brownish (Dresden Brown, pl. XV). SMELL hardly perceptible, fungoid. TASTE mild. SPORE PRINT not obtained.

BASIDIOSPORES [40/1/1] $(9.8\text{--})11.2 \pm 0.8(-13.1) \times (4.0\text{--})4.6 \pm 0.2(-5.0) \mu\text{m}$, $Q = (2.17\text{--})2.22\text{--}2.72(-2.75)$, $Q_m = 2.44 \pm 0.14$, $V = 126 \pm 20 \mu\text{m}^3$, inequilateral, ellipsoid-fusiform to fusiform in side view, ellipsoid-fusiform in face view, smooth under the light microscope, with a prominent apiculus and shallow suprahilar depression, apex rounded, moderately thick-walled ($0.5\text{--}0.7 \mu\text{m}$), straw yellow to bright yellow colored in KOH, having one or (less frequently) two large oil droplets when mature, rarely pluri-guttulate, inamyloid to faintly dextrinoid, cyanophilic and with an orthochromatic reaction; no anomalous spores observed. BASIDIA $28\text{--}37(-40) \times 9\text{--}11 \mu\text{m}$ ($n = 10$), cylindrical-clavate to clavate, moderately thick-walled ($0.5\text{--}0.8 \mu\text{m}$), predominantly 4-spored but

also 2- or (sporadically) 3-spored, usually bearing relatively long sterigmata (2–7 μm), hyaline to very pale yellowish and containing straw-yellow oil guttules in KOH, bright yellow to orange-reddish (barely dextrinoid) in Melzer's, without basal clamps; basidioles subcylindrical to faintly clavate, similar in size to basidia. CHEILOCYSTIDIA (40–)51–76(–82) $\times$ 6–11 μm ($n = 17$), very common, moderately slender, projecting, straight to sometimes flexuous, cylindrical to cylindrical-fusiform, rarely subclavate and occasionally subcapitate or mucronate, with rounded tip, smooth, moderately thick-walled (0.8–1.0 μm), hyaline or with a very pale yellowish pigment in KOH, bright yellow (inamyloid) in Melzer's and with an orthochromatic reaction, without epiparietal encrustations. PLEUROCYSTIDIA (62–)67–110(–113) $\times$ 7–23 μm ($n = 21$), decidedly frequent, color and chemical reactions similar to cheilocystidia but slightly different in shape and distinctly longer, straight, slender, cylindrical-fusiform or fusiform to ventricose-fusiform, less frequently lageniform, sometimes with a narrow and long neck, apex rounded. PSEUDOCYSTIDIA not recorded. PILEIPELLIS a trichoderm or palisadoderm consisting of subparallel to loosely interwoven, elongated, cylindrical, rarely branched hyphae tending to remain erect in the outermost layer and not embedded in gelatinous matter; terminal elements usually long, slender and markedly swollen (cylindrocystes), cylindrical to sausage-shaped, less frequently cystidioid, subclavate or peanut-shaped, acorn-shaped or bullet-shaped, apex rounded-obtuse, (36–)41–88(–103) $\times$ (12–)15–29(–33) μm ($n = 26$), intermixed with scattered longer, filamentous and sinuous cells (up to 127 $\times$ 13 μm), moderately thick-walled ($\leq 1 \mu\text{m}$); pileipellis elements often appear dissociated and easily separable from one another, hyaline to (more often) yellowish in KOH, bright yellow to orange-reddish (weakly dextrinoid) in Melzer's, smooth; subterminal elements similar in shape, size, color and chemical reactions to terminal elements. STIPITPELLIS a texture of slender, subparallel to parallel and longitudinally running, smooth walled, adpressed hyphae, 4–19 μm wide, hyaline in KOH; the stipe apex covered by tufts of caulohymenium consisting of sterile caulobasidioles, very sparse 2-/3-spored fertile caulobasidia similar in shape, size and color to hymenial basidia; CAULOCYSTIDIA abundant, long, subcylindrical to fusiform, ventricose-fusiform or lageniform, projecting, (33–)44–82(–92) $\times$ 7–10(–15) μm ($n = 14$), apex rounded, wall $\leq 1 \mu\text{m}$ thick, hyaline to yellowish in KOH. LATERAL STIPE STRATUM under the caulohymenium absent. STIPE TRAMA hyphae longitudinal, densely arranged, subparallel to moderately interwoven, filamentous, smooth to rarely encrusted by a very subtle granular, brownish pigment, inamyloid to barely dextrinoid, 4–20 μm diam. HYMENOPHORAL

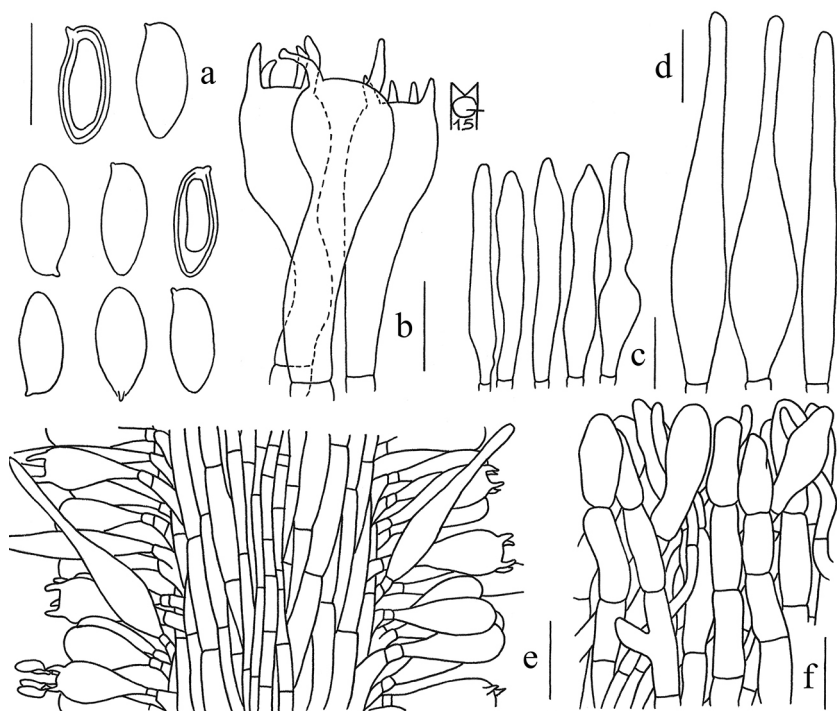


FIG. 4. *Hourangia cheoi* (MG484). a. Basidiospores. b. Basidia. c. Cheilocystidia. d. Pleurocystidia. e. Hymenophoral trama. f. Pileipellis. Scale bars: a, b = 10 µm; c–e = 20 µm; f = 40 µm. Drawings by M. Gelardi.

TRAMA bilateral divergent (“*Phylloporus*-type”), hyphae very slightly divergent to nearly subparallel and tightly arranged, non-gelatinous [lateral strata hyphae in transversal section constricted at septa and distinctly broader than the mediostratum hyphae, touching or almost touching each other, (0–)2–4 µm apart, (7–)10–21(–24) µm diam., relatively thick-walled (≤ 1 µm)], hyaline in KOH, inamyloid in Melzer’s; lateral strata (15–)20–40 µm thick, mediostratum 10–40 µm thick, consisting of a tightly adpressed, non-gelatinous bundle of hyphae, 4–12(–15) µm diam.; in Congo Red the mediostratum is concolorous with the lateral strata. RHIZOMORPHS consisting of parallel and densely arranged, unbranched, cylindrical, moderately thick-walled (≤ 1 µm), smooth hyphae, 5–10(–40) µm diam., yellowish-orange to reddish-brown in KOH, inamyloid to weakly dextrinoid in Melzer’s. CLAMP CONNECTIONS absent in all tissues. HYPHAL SYSTEM monomitic. ONTOGENY gymnocarpic.

ECOLOGY & DISTRIBUTION—Solitary or gregarious on soil in mixed forests dominated by *Fagaceae* (*Quercus*, *Castanopsis*, *Lithocarpus*) and *Pinaceae* (*Keteleeria*, *Pinus*); reported from southwestern China and Japan; distribution limits unknown.

SPECIMEN EXAMINED: CHINA, YUNNAN PROVINCE, Xiaguan County, Cangshan Mountain National Geopark, Gantong Temple, 25°39'03"N 100°09'58"E, 2260 m a.s.l., on a slope facing east in a subtropical montane forest hosting a country graveyard, a single mature specimen with *Pinus yunnanensis* Franch. and *P. armandii* Franch. with the presence of *Quercus variabilis* Blume, 19 Sep 2012, E. Horak & M. Gelardi (E. Horak 13448 (herb. ZT), MG484; GenBank KX911873, KX911874).

ASSOCIATED FUNGI: Other fungal species found in the same habitat are *Amanita parvipantherina* Zhu L. Yang et al. and *Cantharellus appalachiensis* R.H. Petersen.

Discussion

The combination of morphological and molecular analyses confirmed our identification of *Hourangia cheoi*. This eye-catching species appears to be well outlined, especially due to the recent re-description provided by Zhu et al. (2015). Although somewhat variable in its appearance, *H. cheoi* is easily recognized in the field based on the following set of macroscopic characters: its 1) small to medium stature, 2) finely squamulose, ochraceous to brownish or reddish-brown pileus surface that slowly darkens when handled, 3) hymenophore that quickly blues when injured, 4) ≤ 2 mm diam. angular pores, 5) brownish to pale ochraceous fibrillose stipe that grades into beige towards the base, and 5) whitish context that in the pileus turns light blue, then pinkish-red, and finally grayish or dirty brown but in the stipe discolours directly reddish then brownish or grayish-black upon exposure. Diagnostic anatomical features include the 1) medium-sized boletoid basidiospores with a bacillate surface under SEM, 2) very large ($\leq 113 \times 23$ μ m) prominent pleurocystidia, and 3) trichodermal pileipellis comprising swollen cylindrical to sausage-shaped terminal cells up to 33 μ m diam. (Chiu 1948, Zhu et al. 2015; pers. obs.).

Several accounts have treated extensively or cited *Hourangia cheoi* under the specific epithets “cheoi” or “punctilifer” (Zang 1986, 1996, 2006; Li & Song 2000, 2003; Wang 2004, Zhang et al. 2012, Zang et al. 2013), and it has generally been regarded as edible (Li & Song 2002, Dai et al. 2010), albeit in most cases the real identity of the mushroom in cited reports cannot be established with certainty.

The type species of *Xerocomus*, *X. subtomentosus* (L.) Quél., differs from *Hourangia cheoi* by its finely tomentose pileus, pale yellowish context with

pinkish hues in the lower third of the stipe and which turns light blue in the pileus and pileus/stipe connection zone but without an eventual reddish to brownish discoloration, stipe usually coarsely ribbed or roughly pseudoreticulate, trichodermal pileipellis consisting of narrow filamentous hyphae (terminal elements averaging $40 \times 12 \mu\text{m}$), obviously smaller pleurocystidia, temperate distribution, and obligate ectomycorrhizal association with broadleaved trees (mainly *Fagaceae* and *Corylaceae*) (Alessio 1985, Breitenbach & Kränzlin 1991, Engel et al. 1996, Lannoy & Estadès 2001, Ladurner & Simonini 2003, Watling & Hills 2005, Taylor et al. 2006, Klofac 2007, Šutara et al. 2009, Knudsen & Taylor 2012, Galli 2013).

For morphological comparisons of *H. cheoi* with the other three *Hourangia* species, consult the dichotomous key in Zhu et al. (2015).

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